



Research Article

Determining the Biomass Production Capacities of Certain Forage Grasses and Legumes and Their Mixtures under Mediterranean Regional Conditions

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ABSTRACT

Mixtures of annual forage legumes with annual grass for forage and green manure were practiced traditionally in the Mediterranean conditions. The aim of the present study was to determine of biomass production capacities of three different grasses species [wheat (*Triticum aestivum* L.), triticale (*Triticosecale* Witt.), annual ryegrass (*Lolium multiflorum* L.)], two legumes forages [common vetch (*Vicia sativa* L.), berseem clover (*Trifolium alexandrinum* L.)] and their mixtures (75% legume+25% grass). The field experiments were conducted at East Mediterranean Region of Turkey during 2001-02 and 2002-03 growing seasons. The results of study showed that yields of mixtures were higher than sole sowings. According to results; the highest green herbage yield was obtained from common vetch+wheat mixture with 66.2 t ha⁻¹, while the lowest was obtained from sole wheat with 23.2 t ha⁻¹. The superior nitrogen ratio in hay (3.09%) and in dry root (2.48%) values were determined in common vetch; the highest nitrogen yield in hay (474 kg ha⁻¹) and in dry biomass (510 kg ha⁻¹) values were obtained from berseem clover. The highest fresh root yield (18.1 t ha⁻¹), dry root yield (3.7 t ha⁻¹) and nitrogen yield in dry root (71.3 kg ha⁻¹) values were obtained from annual ryegrass. The superior crude ash ratio was obtained at 11.2% from common vetch and the highest crude ash ratio in dry root (37.3%) was obtained from berseem clover+wheat mixture. In terms of total biomass production capacity, the common vetch+wheat, the berseem clover+triticale and the berseem clover+wheat mixtures provide the best results; in point of fresh and dry root residues deposited per unit area, wheat, annual ryegrass and triticale stand out, while the berseem clover+triticale and the berseem clover+annual ryegrass mixtures also deposit an abundance of root residues.

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Introduction

The increase in the world population and the improvement of industrialization led to an influx problem, such as food insecurity, hunger and malnutrition for millions of people, concerning human health and environment (Varshney *et al.* 2011). One of the major problems is brought on by the use of excessive fertilizers and pesticides to obtain higher productivity. Both fertilizers and pesticides increase productivity of agricultural production but the chemical residue influences human health (Demirkol *et al.* 2012). Pasture lands and the growing of forages not only provide balanced, quality and low-cost sources of fodder for livestock, but they also include plants that can help in re-planning of agricultural lands for proper and applicable use, improvement of lands with soils having low levels of organic matter, and protection of areas subjected to erosion (Saglamtimur *et al.* 1998; Acikgoz 2001). In addition to biologically derived food, as well as fruits and vegetables consumed directly by humans, the application of organic farming should also be considered within the scope of livestock health and animal products.

Organic material obtained by growing, protecting and using grass acquired from continuously used pastures and temporarily formed pastures lead to improvements in livestock health and increases in livestock productivity. Moreover, use of improved cultivars, with the appropriate management, can add value to animal products (Woodfield and Easton 2004). Biomass is defined as the sum total of the plant component above and below the soil line and is an important concept, which directly affects soil productivity. The integration of cover crops into cropping systems brings costs and benefits, both internal and external to the farm (Snapp *et al.* 2005). Soil productivity is the ability, under the effect of specific chemical, physical and biological factors, to provide the required nutrients and water to the plant cover, and especially to the higher plants, found within the composition of the soil (Eldor 2007).

Green manure, which is the incorporation of crop biomass to soil, is a topic of high interest for biological farming and its approaches to crop production might improve economic viability, while reducing the environmental impacts of agriculture. Because the application of green manure minimizes, and in fact removes, the use of synthetic fertilizers, it plays a vital role in the production of biological nutrients and reduction of costs (Cher *et al.* 2006). When the use of green manure is supported with farming fertilizers, biological nutrients may be produced for cheaper than when using synthetic chemicals and nutrients that are harmful to health.

One of the best ways of enriching the organic matter of soil is through the cultivation of forages, as well as crop rotation applied within the system of field agriculture. In forage crops production, many intercropping systems are used for different aim. Thanks to these systems it is possible to increase the yield and quality obtained per unit area (Acar *et al.* 2006). Forage legumes have significant role in agriculture and are often integrated in mixed farming systems to enhance crop yields through their nitrogen fixing ability as well as their use for high quality feed for intensive livestock systems (Anwar *et al.* 2010). Each year, at global level, in the agroecosystems, about 50 million t of nitrogen are fixed (Herridge *et al.* 2008). Likewise, Unkovich *et al.* (2008) consider that legume species fix between 15 and 25 kg nitrogen t⁻¹ of dry matter.

In this sense, forage legumes are a step ahead of, and more favored than, forage grasses, due to their prevention of the rapid wash out of nitrogen, quick decomposition and degradation when incorporated timely into the soil, ability to deposit generous amounts of plant residue and to help form good soil structure due to their deep reaching root systems. Besides, major benefits of increasing the soil organic N levels through green manure is an increase in the mid growing season N mineralization, which in most cases translates into a higher grain N content (Olesen *et al.* 2009).

When crops are grown in the Mediterranean climate conditions, cultivated for green manure, they can be harvested 25 to 30 days prior to the harvesting of the main crop during grain formation at the start of the spring season, and incorporated into the soil (Saglamtimur *et al.* 1998). Therefore, cultivation of legumes as mixtures with cereals or grasses and their use within crop rotation will not only enrich the soil in terms of organic matter, but will also help in physical and chemical fortification of the soil.

The objective of this study was to determine the biomass production capacities for certain forages grasses and legumes when cultivated as mixtures, as well as to determine the levels of beneficial effects they have on soil productivity.

Materials and Methods

Three species of *Poaceae*, wheat (*Triticum aestivum* L.), triticale (*Triticosecale* Witt.) and annual ryegrass (*Lolium multiflorum* L.), and two species of *Fabaceae*, common vetch (*Vicia sativa* L.) and berseem clover (*Trifolium alexandrinum* L.), were provided from the Field Crops Department of Agricultural Faculty of Cukurova University.

This study has been conducted at a bottomland (37°57'N and 35°30'E, altitude 24 m) located at the Research and Implementation Area of the Faculty of Agriculture's Department of Field Crops at Cukurova University, during the 2001-02 and 2002-03 growing periods, using a randomized complete block design with 3 replications. Sole cultivation of legumes and their 75%-25% mixture with grasses; make up the 11 systems used in the study. Due to legumes having the characteristics of nitrogen fixation, roots that extend deep into the soil, leaving root residues in the soil, abundance of above-ground parts, as well as to grasses having the characteristic of not deluging legumes during tillering when cultivated

as a mixture, the ratio of legumes has been kept high in the mixture.

The size of each plot was 8.80 m² (2.20x4.0 m). During cultivation, the following amounts of seeds have been used: 600 plants m⁻² to obtain 200 kg ha⁻¹ for wheat and triticale, 45 kg ha⁻¹ for annual ryegrass, 120 kg ha⁻¹ for common vetch, and 20 kg ha⁻¹ for berseem clover. For both years, sowing realized in the first week of November. For sole stands of wheat, triticale, annual ryegrass, 100 kg ha⁻¹ of starter fertilizer have been used; on the other hand, for the mixtures of common vetch and berseem clover, 50 kg ha⁻¹ of 20-20-0 starter fertilizer has been used in sole N and P₂O₅ form.

The harvesting and root sampling of the above-ground parts have been carried out during the same month; harvesting for the first year was realized during the last week of April when the lower pods of common vetch begin to set and the full blossoming of the berseem clover takes place; harvesting for the second year was performed during the 2nd week of April, corresponding to the same stage in the growth of the plants. Green herbage and fresh root samples were collected from 10 randomly selected separate locations in each plot that were free of edge effect, using an Albrecht Bohrer auger drill with a 16 cm diameter and a 30 cm length. Green herbage and fresh root samples, 500 g in weight, obtained from each plot were dried at 70 °C for a period of 24 hours, following which the weights of the hay and dried roots were determined.

Afterwards, the hay and dry root samples were ground in a hand-mill with a 1 mm diameter filter. Kjeldahl method was used to determine nitrogen (N) contents of dry samples (AOAC, 1990). The ratios thus obtained were multiplied with the yield values for hay and dry roots. Crude ash contents of the samples were determined by burning the samples at 550 °C for 12 hours.

During the growing period when the trial was made, average temperature values followed a course parallel to average values for long years, with no observation of any value that would negatively affect the development path of plants. It was found that the rainfall was irregular in consideration of both the averages of the trial years and of long years Table 1.

Soils where the study was conducted are entisols brought by Seyhan River, formed of very young alluvial deposits. They are in almost flat and near-flat topographies. There are only A-horizons well-decomposed by external influences and rich in organic matters and humus and C-horizons formed of large pieces and located over the main rock. The trial area soil was generally loam.

Analysis of variance of the experimental results was performed by using MSTAT-C statistical software and Duncan's multiple range tests was employed to compare the treatment means Table 2.

Results

The results of the variance analysis showed that the effects of the plant and mixtures on the whole properties, which is plant height, green herbage yield, hay yield, fresh root yield, dry root yield, nitrogen ratio in hay, nitrogen ratio in dry root, nitrogen yield in hay, nitrogen yield in dry root, crude ash ratio, crude ash root in dry root, fresh biomass yield, dry biomass yield, nitrogen yield in dry biomass were significant Table 2. According to the Table 2, there is a 1% statistically significant difference among the all properties.

Upon analyzing Table 3 and 4 where two-year average plant heights and green herbage yields

values are presented, it is observed that average plant height values are in the range from 84.0 to 141.3 cm, while green herbage yields vary between 23.2 and 66.2 t ha⁻¹. The lowest hay yield value of 6.4 t ha⁻¹ were obtained from annual ryegrass and wheat, that is statistically in the same group; the highest hay values were in common vetch+wheat mixtures and berseem clover+triticale mixtures (16.4 t ha⁻¹) and, as well as for the berseem clover (16.1 t ha⁻¹), which for statistical purposes, is contained in the same group. In case of fresh root yield, the minimum value obtained was 5.9 t ha⁻¹ for common vetch and the maximum value was 18.1 t ha⁻¹ for the annual ryegrass.

The lowest dry root yield value of 1.09 t ha⁻¹ was obtained in common vetch; the highest dry root values (3.70 t ha⁻¹) were for annual ryegrass and, as well as for the triticale (3.66 t ha⁻¹) which, for statistical purposes, is contained in the same group. In case of nitrogen ratio in hay, the lowest value obtained were 2.25% for common vetch+wheat mixtures and, as well as for the triticale and wheat (2.33%) which, for statistical purposes, is contained in the same group; the highest value was 3.09% for the common vetch.

The minimal nitrogen ratio value in dry root of 1.51% were obtained for berseem clover+wheat mixture, berseem clover+triticale and common vetch+wheat (1.53%) mixtures; the maximal nitrogen ratio values in dry root (2.48%) were for common vetch and, as well as for the sole berseem clover (2.43%) which, for statistical purposes, is contained in the same group. In case of nitrogen yield in hay, the lowest value obtained were 156 kg ha⁻¹ for common wheat and, as well as for the annual ryegrass (160 kg ha⁻¹) and common vetch+annual ryegrass (161 kg ha⁻¹) mixtures which, for statistical purposes, are contained in the same group; the highest value was 474 kg ha⁻¹ for the berseem clover.

Table 1. Some climatic data in the experimental area

Months	Temperature (°C)			Precipitation (mm)		
	1 st year	2 nd year	Long Period	1 st year	2 nd year	Long Period
November	13.9	16.4	15.3	88.1	25.7	78.4
December	10.7	8.8	10.9	320.9	77.9	121.2
January	7.9	11.1	9.6	109.2	84.5	101.6
February	12.3	8.2	10.5	68.1	111.7	83.3
March	14.7	11.5	13.6	40.3	92.3	60.2
April	16.5	17.1	17.6	88.8	61.1	57.7
May	21.4	24.5	21.8	22.0	14.8	42.5
Average	13.9	13.9	14.2	-	-	-
Total	-	-	-	737.2	468.0	544.9

Table 2. Summary of ANOVA of the traits determined based on the combined analysis over two-years.

Source of Variation	DF	PH	GHY	HY	FRY	DRY	HNR	DRNR
Year (Y)	1	ns	ns	ns	ns	ns	**	*
Plant and Mixtures (PM)	10	**	**	**	**	**	**	**
YxPM	10	**	**	**	**	**	**	**
Source of Variation	DF	HNY	DRNY	CAR	DRCAR	FBY	DBY	DBNY
Year (Y)	1	**	ns	*	*	ns	Ns	**
Plant and Mixtures (PM)	10	**	**	**	**	**	**	**
YxPM	10	**	**	**	**	**	**	**

*Abbreviations: DF: Degrees of freedom, NS: Not significant, *P < 0.05, **P < 0.01, PH: Plant height, GHY: Green herbage yield, HY: Hay yield, FRY: Fresh root yield, DRY: Dry root yield, HNR: Nitrogen ratio in hay, DRNR: Nitrogen ratio in dry root, HNY: Nitrogen yield in hay, DRNY: Nitrogen yield in dry root, CAR: Crude ash ratio, DRACR: Crude ash ratio in dry root, FBY: Fresh biomass yield, DBY: Dry biomass yield, DBNY: Nitrogen yield in dry biomass

While the lowest nitrogen yield value in dry root of 21.5 kg ha⁻¹ was obtained for common vetch+wheat mixture; the highest nitrogen yield values in dry root 71.3 kg ha⁻¹ was for the annual ryegrass. In terms of crude ash ratio in hay, the minimum value obtained was 6.2% for berseem clover+annual ryegrass; the maximum value was 11.2% for the common vetch. While the fewest crude ash ratios in dry root were obtained at 14.0% in the common vetch+wheat mixture, as well as from common vetch, statistically located in the same group (14.8%); the utmost crude ash ratio in dry root was obtained at 37.3% in the berseem clover+ wheat mixture.

In terms of fresh biomass yield, the lowest value was determined for wheat at 30.6 t ha⁻¹, and the highest value at 75.6 t ha⁻¹ for the berseem clover+triticale mixture. The lowest dry biomass yield was obtained at 7.8 t ha⁻¹ from the common vetch+annual ryegrass mixture; the highest dry biomass yield was obtained at 18.9 t ha⁻¹ from the berseem clover+triticale mixture. In terms of nitrogen yield in dry biomass, the lowest values were determined for common vetch+annual ryegrass mixture (193 kg ha⁻¹) and common wheat (207 kg ha⁻¹), and the highest value at 510 kg ha⁻¹ for the berseem clover.

When the results are analyzed for fresh root yields, it is observed that the annual ryegrass from the *Poaceae* family stands out compared to sole stands of other species. As is known, the species in the *Poaceae* family have fibrous, and the species in the *Fabaceae* family have taproot/deeproot, root systems. Grasses have roots that show more of a lateral development; even in those species where roots extend deep, a majority of the roots are concentrated in the upper segments of the soil located 0 to 20 cm (Saglamtimur *et al.* 1998; Acikgoz 2001).

The similarity of dry root yields, obtained from fresh root samples with the water removed from the root, to those of fresh root yields, may also be considered an expected outcome, much like in the

case of the green herbage/dry fodder relationship. There are numerous studies assessing the amount of fixed nitrogen, particularly of symbiotic nitrogen from the relation of nitrogen-fixing bacteria and species of the Family *Fabaceae*, depending on the ecological area and on the cultivation technology (Ghiocel *et al.* 2012). Therefore it may be concluded that the ratio of nitrogen obtained from legumes such as common vetch and berseem clover, being higher compared to that of the *Poaceae* family, is the result of this characteristic. Indeed, researchers have shown (Sparrow and Masiak 2004; Budak 2005; Turgut *et al.* 2006; Javanmard *et al.* 2009), in studies conducted that in addition to higher levels of nitrogen detected in legumes compared to that in grasses, the ratio of nitrogen obtained from mixtures decreased in relation to the increasing grass ratios in these mixtures.

Also, legumes accumulate the free nitrogen from the air in various organs of the plant, while using the roots for supplementary storage of nutrients. The effects of legumes were usually considered to be a direct result of higher N availability (Nyfeler *et al.* 2011). As a result of this situation, the amount of nitrogen accumulated in roots for common vetch and berseem clover, species of legumes, are observed to be higher than that observed for other species. At the same time, the lower levels of nitrogen obtained from the mixture of these two species with grasses, may be explained by the higher numbers of roots formed by grasses due to their dominance over legumes in the mixtures.

Table 3. Plant height, green herbage yield, hay yield, fresh root yield, dry root yield, nitrogen ratio in hay and nitrogen ratio in dry root values of sole sowings and mixtures. Data are the means of 2 years.

Plants and Mixtures	PH (cm)	GHY (t ha ⁻¹)	HY (t ha ⁻¹)	FRY (t ha ⁻¹)	DRY (t ha ⁻¹)	HNR (%)	DRNR (%)
Wheat	84.0 g	23.2 i	6.7 f	7.9 f	2.52 c	2.33 e	1.93 c
Annual ryegrass	106.5 f	45.3 g	6.4 f	18.1 a	3.70 a	2.47 d	1.93 c
Triticale	119.5 de	46.3 g	12.3 c	14.0 b	3.66 a	2.33 e	1.86 d
Common vetch	121.9 d	53.9 f	10.0 e	5.9 i	1.09 f	3.09 a	2.48 a
Berseem clover	141.3 a	56.3 d	16.1 a	6.5 h	1.28 e	2.96 b	2.43 a
Common vetch 75%+Annual ryegrass 25%	134.9 b	35.6 h	6.0 g	9.5 e	2.02 d	2.70 c	1.59 e
Common vetch 75%+Wheat 25%	116.8 e	66.2 a	16.4 a	8.0 f	1.41 e	2.25 e	1.53 f
Common vetch 75%+Triticale 25%	133.3 b	54.1 ef	11.7 d	6.8 g	1.89 d	2.64 c	2.28 b
Berseem clover 75%+Annual ryegrass 25%	128.7 c	55.5 de	12.2 cd	11.8 c	2.69 b	2.43 d	1.98 c
Berseem clover 75%+Wheat 25%	140.3 a	58.3 c	15.4 b	10.7 d	2.57 bc	2.52 d	1.51 f
Berseem clover 75%+Triticale 25%	119.1 de	61.4 b	16.4 a	14.3 b	2.55 bc	2.43 d	1.53 f
Mean	122.4	50.6	11.8	10.3	2.31	2.56	1.91
CV (%)	2.20	2.33	3.99	2.81	5.34	3.13	2.51

*Different letters in a column indicate significant difference at $P < 0.05$; C.V.: Coefficient of variation.

Table 4. Nitrogen yield in hay, nitrogen yield in dry root, hay crude ash ratio, crude ash ratio in dry root, fresh biomass yield, dry biomass yield, nitrogen yield in dry biomass values of sole sowings and mixtures. Data are the means of 2 years.

Plants and Mixtures	HNY (kg ha ⁻¹)	DRNY (kg ha ⁻¹)	HCAR (%)	DRCAR (%)	FBY (t ha ⁻¹)	DBY (t ha ⁻¹)	DBNY (kg ha ⁻¹)
Wheat	156 e	50.8 c	8.3 c	28.5 c	30.6 h	9.3 h	207 g
Annual ryegrass	160 e	71.3 a	10.2 b	31.2 b	63.4 e	10.1 g	231 f
Triticale	289 d	67.8 b	7.2 e	31.5 b	60.3 f	16.0 c	356 d
Common vetch	307 d	27.1 g	11.2 a	14.8 f	59.8 f	11.0 f	335 e
Berseem clover	474 a	31.2 f	7.5 de	18.7 e	62.7 e	17.4 b	510 a
Common vetch 75%+Annual ryegrass 25%	161 e	32.0 f	8.0 cd	17.7 e	45.1 g	7.8 i	193 g
Common vetch 75%+Wheat 25%	370 c	21.5 h	7.5 de	14.0 f	74.2 b	17.8 b	391 c
Common vetch 75%+Triticale 25%	309 d	42.7 d	10.0 b	23.3 d	61.0 f	13.6 e	352 de
Berseem clover 75%+Annual ryegrass 25%	297 d	53.2 c	6.2 f	23.0 d	67.3 d	14.9 d	350 de
Berseem clover 75%+Wheat 25%	390 b	39.2 e	7.3 de	37.3 a	69.0 c	17.5 b	428 b
Berseem clover 75%+Triticale 25%	398 b	38.8 e	6.8 ef	22.8 d	75.6 a	18.9 a	437 b
Mean	301	43.2	8.20	23.9	60.8	14.0	344
CV (%)	5.32	6.19	7.66	5.71	1.83	4.44	4.82

*Different letters in a column indicate significant difference at $P < 0.05$; C.V.: Coefficient of variation.

Soils with a higher nutrient supply capacity require reduced fertilizer inputs. If fertilizer costs are reduced while yield is maintained, profitability over the long-term may more than compensate the immediate costs of cover crop establishment (Snapp *et al.* 2005). In other researchers (Serin *et al.* 1999; Hatipoglu *et al.* 2001; Sheaffer *et al.* 2001; Ross *et al.* 2004; Budak 2005; Fulkersen *et al.* 2007; Nykanen *et al.* 2009; Lithourgidis *et al.* 2011; Nyfeler *et al.* 2011) have been determined that both legumes, and mixtures containing legumes, dispose varying levels of nitrogen in the soil based on the plant species, mixture ratios, as well as the yields obtained.

It is noteworthy that contrary to such species of legumes as common vetch and berseem clover, which accumulate more nitrogen in their roots, higher values of nitrogen levels in dry root are obtained from grasses such as annual ryegrass and triticale. But as explained earlier and also observed from the values obtained, the overall higher root yields from grasses have had an effect in obtaining a higher value for nitrogen content in dry root. In plant species incinerated at 550 °C whose organic matter has completely evaporated and whose minerals have remained, a higher ratio of raw ash (remaining in the roots) has been obtained compared to the raw ash ratio obtained from dry fodder. In their study conducted in 2006, they have determined an average raw ash ratio in the range of 9.1 to 10.0%, and have additionally reported that this ratio has decreased as the plant development stage has progressed (Ozyigit and Bilgen 2006).

While fresh biomass may show statistical variations among species, mostly similar values have been obtained for species except for wheat and for the common vetch+annual ryegrass mixtures, which displayed significantly lower values. However, it was observed that the berseem clover+triticale and the common vetch+wheat mixtures, for which the highest values have been obtained, slightly stand out.

In addition to high ratios of nitrogen obtained from the species, the ability of the plants to leave behind generous amounts of above ground and below ground residues, is a sought after characteristic. This is due to the fact that the main source of soil organic matter is plant residues and achieving organic matter amounts at a desired level is an indication of soil that is biologically, physically and chemically improving. In this study, high ratios of nitrogen yields have been obtained from sole berseem clover, as well as from its mixtures with various grasses.

Conclusion

According to results; in terms of total biomass production capacity, the common vetch+wheat, the berseem clover+triticale and the berseem clover+wheat mixtures provide the most convenient results; in point of fresh and dry root residues deposited per unit area, wheat, annual ryegrass and triticale stand out, while the berseem clover+triticale and the berseem clover+annual ryegrass mixtures also deposit an abundance of root residues.

In recognition of the element nitrogen that is important for feeding livestock, replenishing organic matter in the soil, and improving the yields of subsequently planted crops, common vetch and berseem clover, as well as the berseem clover+triticale and the berseem clover+wheat mixtures provide the most appropriate results.

When analyzed from the perspective of providing balanced and palatable fodder for livestock and of providing sufficient organic matter for maintaining long durations and high levels of soil improvement and productivity, it has been concluded that the mixtures of berseem clover+annual ryegrass and triticale should be addressed with priority due to the high amounts of biomass they produce, while at the same time providing the soil with nitrogen, and that it would make for a suitable application to grow legumes

in sole stands or as mixtures and incorporate them into the soil as green manure.

Additionally, while it may be seem to be a positive outcome for annual ryegrass and triticale to deposit generous amounts of root residue, due to having fibrous root structures and being from the *Poaceae* family, it is recommended that their mixed cultivation with legumes should be preferred over their solitary use as in such use they will not be able to contribute to soil replenishment at sufficient levels.

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